

01-27-00

A

Please type a plus sign (+) inside this box → ☒Approved for use through 09/30/2000. OMB 0651-0032
Patent and Trademark Office, U.S. DEPARTMENT OF COMMERCE

Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

UTILITY PATENT APPLICATION TRANSMITTAL (Only for new nonprovisional applications under 37 C.F.R. § 1.53(b))	Attorney Docket No.	UF-232XC1
	First Inventor or Application Identifier	Jane E. Polston
	Title	Materials and Methods for Producing Geminivirus Resistant Plants
	Express Mail Label No.	EK318905915US

APPLICATION ELEMENTS See MPEP chapter 600 concerning utility patent application contents.	ADDRESS TO: Assistant Commissioner for Patents Box Patent Application Washington, DC 20231	
1. <input checked="" type="checkbox"/> * Fee Transmittal Form (e.g., PTO/SB/17) (3 pages) (Submit an original and a duplicate for fee processing)	5. <input type="checkbox"/> Microfiche Computer Program (Appendix)	
2. <input checked="" type="checkbox"/> Specification [Total Pages 21] - Descriptive title of the Invention - Cross References to Related Applications - Statement Regarding Fed sponsored R & D - Reference to Microfiche Appendix - Background of the Invention - Brief Summary of the Invention - Brief Description of the Drawings (if filed) - Detailed Description - Claim(s) - Abstract of the Disclosure	6. Nucleotide and/or Amino Acid Sequence Submission (if applicable, all necessary) a. <input type="checkbox"/> Computer Readable Copy b. <input type="checkbox"/> Paper Copy (identical to computer copy) c. <input type="checkbox"/> Statement verifying identity of above copies	
3. <input checked="" type="checkbox"/> Drawing(s) (35 U.S.C. 113) [Total Sheets 3]	ACCOMPANYING APPLICATION PARTS 7. <input type="checkbox"/> Assignment Papers (cover sheet & document(s)) 8. <input type="checkbox"/> 37 C.F.R. § 3.73(b) Statement of Power of Attorney (when there is an assignee) 9. <input type="checkbox"/> English Translation Document (if applicable) 10. <input type="checkbox"/> Information Disclosure Statement (IDS)/PTO-1449 [Copies of IDS Citations] 11. <input type="checkbox"/> Preliminary Amendment 12. <input checked="" type="checkbox"/> Return Receipt Postcard (MPEP 503) (Should be specifically itemized) 13. <input checked="" type="checkbox"/> * Small Entity Statement(s) [Statement filed in prior application, Status still proper and desired (PTO/SB/09-12)] 14. <input type="checkbox"/> Certified Copy of Priority Document(s) (if foreign priority is claimed) 15. <input type="checkbox"/> Other: _____	
4. Oath or Declaration [Total Pages 2] a. <input checked="" type="checkbox"/> Newly executed (original or copy) (unsigned) b. <input type="checkbox"/> Copy from a prior application (37 C.F.R. § 1.63(d)) (for continuation/divisional with Box 16 completed) i. <input type="checkbox"/> DELETION OF INVENTOR(S) Signed statement attached deleting inventor(s) named in the prior application, see 37 C.F.R. §§ 1.63(d)(2) and 1.33(b).		
* NOTE FOR ITEMS 1 & 13: IN ORDER TO BE ENTITLED TO PAY SMALL ENTITY FEES, A SMALL ENTITY STATEMENT IS REQUIRED (37 C.F.R. § 1.27), EXCEPT IF ONE FILED IN A PRIOR APPLICATION IS RELIED UPON (37 C.F.R. § 1.28).		

16. If a CONTINUING APPLICATION, check appropriate box, and supply the requisite information below and in a preliminary amendment:
☐ Continuation ☐ Divisional ☐ Continuation-in-part (CIP) of prior application No: _____
Prior application information: Examiner _____ Group / Art Unit _____
For CONTINUATION or DIVISIONAL APPS only: The entire disclosure of the prior application, from which an oath or declaration is supplied under Box 4b, is considered a part of the disclosure of the accompanying continuation or divisional application and is hereby incorporated by reference. The incorporation can only be relied upon when a portion has been inadvertently omitted from the submitted application parts.

17. CORRESPONDENCE ADDRESS					
<input type="checkbox"/> Customer Number or Bar Code Label [] or <input checked="" type="checkbox"/> Correspondence address below (Insert Customer No. or Attach bar code label here)					
Name	Doran R. Pace Saliwanchik, Lloyd & Saliwanchik				
Address	2421 N.W. 41st Street Suite A-1				
City	Gainesville	State	FL	Zip Code	32606
Country	USA	Telephone	(352) 375-8100	Fax	(352) 372-5800

Name (Print/Type)	Doran R. Pace	Registration No. (Attorney/Agent)	38,261
Signature		Date	Jan. 25, 2000

Burden Hour Statement: This form is estimated to take 0.2 hours to complete. Time will vary depending upon the needs of the individual case. Any comments on the amount of time you are required to complete this form should be sent to the Chief Information Officer, Patent and Trademark Office, Washington, DC 20231. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: Assistant Commissioner for Patents, Box Patent Application, Washington, DC 20231.

Applicant or Patentee: Jane E. Polston, Ernest Hiebert, Ahmed M. Abouزيد, Wayne Attorney's
Serial or Patent No.: B. Hunter Docket No. UF-232XC1
Filed or Issued: January 25, 2000
For: Materials and Methods for Producing Geminivirus Resistant Plants

VERIFIED STATEMENT (DECLARATION) CLAIMING SMALL ENTITY
STATUS (37 CFR 1.9 (f) and 1.27 (c)) – NONPROFIT ORGANIZATION

I hereby declare that I am an official empowered to act on behalf of the nonprofit organization identified below:

NAME OF ORGANIZATION University of Florida
ADDRESS OF ORGANIZATION 223 Grinter Hall
Gainesville, FL 32611

TYPE OF ORGANIZATION

- ☒ UNIVERSITY OR OTHER INSTITUTION OF HIGHER EDUCATION
☐ TAX EXEMPT UNDER INTERNAL REVENUE SERVICE CODE (26 USC 501(a)(3))
☐ NONPROFIT SCIENTIFIC OR EDUCATIONAL UNDER STATUTE OF STATE OF THE UNITED STATES OF AMERICA
(NAME OF STATE _____)
(CITATION OF STATUTE _____)
☐ WOULD QUALIFY AS TAX EXEMPT UNDER INTERNAL REVENUE SERVICE CODE (26 USC 501(a) and 501(c)(3)) IF LOCATED IN THE UNITED STATES OF AMERICA
☐ WOULD QUALIFY AS NONPROFIT SCIENTIFIC OR EDUCATIONAL UNDER STATUTE OF STATE OF THE UNITED STATES OF AMERICA IF LOCATED IN THE UNITED STATES OF AMERICA
(NAME OF STATE _____)
(CITATION OF STATUTE _____)

I hereby declare that the above identified nonprofit organization qualifies as a nonprofit organization as defined in 37 CFR 1.9 (d), for purposes of paying reduced fees under section 41(a) and (b) of Title 35, United States Code, with regard to the invention described in the above-identified:

☐ PATENT ☒ APPLICATION

I hereby declare that rights under contract or law have been conveyed to and remain with the nonprofit organization identified above with regard to the above-identified invention.

If the rights held by the above identified nonprofit organization are not exclusive, each individual, concern or organization having rights to the invention is listed below* and no rights to the invention are held by any person, other than the inventor, who could not qualify as a small business concern under 37 CFR 1.9 (d) or by any concern which would not qualify as a small business concern under 37 CFR 1.9 (d) or a nonprofit organization under 37 CFR 1.9 (e).

*NOTE: Separate verified statements are required from each named person, concern or organization having rights to the invention averring their status as small entities. (37 CFR 1.27)

NAME _____
ADDRESS _____
☐ INDIVIDUAL ☐ SMALL BUSINESS CONCERN ☐ NONPROFIT ORGANIZATION

NAME _____
ADDRESS _____
☐ INDIVIDUAL ☐ SMALL BUSINESS CONCERN ☐ NONPROFIT ORGANIZATION

I acknowledge the duty to file, in this application or patent, notification of any change of status resulting in loss of entitlement to small entity status prior to paying, or at the time of paying, the earliest of the issue fee or any maintenance fee due after the date on which status as a small entity is no longer appropriate. (37 CFR 1.28 (b))

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application, any patent issuing thereon, or any patent to which this verified statement is directed.

NAME OF PERSON SIGNING Thomas E. Walsh, Ph.D.
TITLE IN ORGANIZATION Director of Sponsored Research
ADDRESS OF PERSON SIGNING 223 Grinter Hall
Gainesville, FL 32611

SIGNATURE Tw Walsh DATE January 25, 2000

DESCRIPTION

MATERIALS AND METHODS FOR PRODUCING GEMINIVIRUS RESISTANT PLANTS

5

The subject invention was made with government support under a research project supported by USDA Grant No. 92341357456 and USDA Grant No. 98341356784. The government has certain rights in this invention.

10

Cross-Reference to a Related Application

This application claims the benefit of U.S. Provisional Application No. 60/117,151 filed January 25, 1999.

Background of the Invention

15

Whitefly-transmitted geminiviruses have become a major limiting factor in tomato production in Florida, the Caribbean and much of Latin America. This group of viruses is currently expanding in the Western Hemisphere, and the number of characterized geminiviruses which infect tomato in this region has increased from three to more than 17 over the last 10 years (Polston and Anderson, 1997). This expansion is continuing and reports of new epidemics are appearing almost monthly. Whitefly-transmitted viruses appear alone and in mixed infections with other geminiviruses and other viruses. Whitefly-transmitted geminiviruses are reducing tomato yields in many countries, and total crop losses are not uncommon (Polston and Anderson, 1997). Tomato production in Florida has suffered significant losses (estimated at \$125 million in 1990-91) due to tomato mottle virus (ToMoV) infection, which first appeared in 1989. There are no estimates of losses in Puerto Rico due to the tomato geminiviruses, potato yellow mosaic virus (PYMV) and ToMoV, but yields have been reduced significantly (Brown *et al.*, 1995). Tomato yellow leaf curl virus (TYLCV-Is) which caused extensive losses to tomato production in the Dominican Republic

20

25

(reviewed by Polston and Anderson, 1997) has now been found in Florida (Polston *et al.*, 1999). Incidences of TYLCV-Is are increasing and economic losses were felt this past fall (1998). TYLCV-Is is widespread in Florida, is likely to increase over the next few years and will become a major constraint to tomato production in Florida.

5 Geminiviruses are very difficult to economically manage in fresh market tomatoes, and practically impossible to manage in processing tomatoes. At this time geminiviruses are managed primarily through the use of a single insecticide, imidacloprid, to reduce the population of the whitefly vector. Tolerance to this insecticide has already been reported from other countries (Cahill *et al.*, 1996; Williams *et al.*, 1996). It may be only a matter of
10 time before imidacloprid loses efficacy in the United States and other locations. The average Florida tomato grower spent approximately \$250/acre for insecticides to control ToMoV in 1994 through 1997. These costs are expected to increase significantly as growers' struggle to manage TYLCV-Is. In Caribbean countries geminiviruses have caused many small and medium size tomato growers to go out of business due the increases in costs of production and crop losses. In Israel, where imidacloprid resistance is present, TYLCV-Is is managed
15 by pesticide use plus exclusion; tomatoes are produced in greenhouses enclosed in whitefly-proof screening material or in screened tunnels in the field. The use of these methods are expensive and are often not an economically or horticulturally realistic alternative. The least expensive and most practical control of whitefly-transmitted geminiviruses is the use of resistant cultivars. At this time there are no commercially available resistant tomato cultivars
20 for the geminiviruses native to the Western Hemisphere. There are several cultivars available which have tolerance to TYLCV-Is, however the fruit size and the horticultural attributes of these cultivars are unsuitable for production in Florida.

 There are no commercially available ToMoV-resistant tomato cultivars. ToMoV-
25 resistance from *Lycopersicon* species has been incorporated into tomato (*L. esculentum*) backgrounds but resistance is closely linked with small fruit size. This linkage has significantly delayed development of resistant plants. Resistance to ToMoV in both tobacco and tomato has been described using mutated coat protein and movement protein genes from

ToMoV (Abouzid *et al.*, 1996; Duan *et al.*, 1997a; Duan *et al.*, 1997b; Polston *et al.*, 1996; Sinisterra *et al.*, 1997; Sinisterra *et al.*, 1999). A mutated *BC1* gene has been shown to give broad-spectrum resistance (Duan *et al.*, 1997a).

There are few reports suggesting that the gene encoding the geminivirus replication associated protein (Rep) might be used for resistance. There has been a report that a modified ToMoV *Rep* mutated in a NTP-binding motif was transformed into tomato plants and demonstrated to interfere with viral replication (Stout *et al.*, 1997). Hanson *et al.* (1995) analyzed phenotypes of BGMV (bean golden mosaic virus) with mutations in a NTP-binding motif of the *Rep* gene, and demonstrated that the NTP-binding domain is required for replication. They proposed that mutations in this motif may serve in a trans-dominant negative interference scheme for pathogen-derived resistance (also known as “dominant negative mutations”). Resistance to African cassava mosaic geminivirus (ACMV) in *Nicotiana benthamiana* plants was developed by transformation with ACMV *Rep* (Hong and Stanley, 1996).

Resistance has been reported with the *Rep* gene of a monopartite virus, tomato yellow leaf curl virus (TYLCV), a geminivirus only distantly related to ToMoV. Noris *et al.* (1996) found TYLCV-resistance in *N. benthamiana* plants using the TYLCV *Cl* gene with a truncated C-terminal (210 amino acids). However, resistance was overcome with time. Brunetti *et al.* (1997) transformed tomatoes with the same construct and found that high accumulation of the truncated Rep protein was required for resistance, that high accumulation resulted in a “curled” phenotype, and that the resistance did not extend to an unrelated geminivirus. The plants transformed according to the methods of the subject invention have a normal phenotype and are high yielding as well.

Brief Summary of the Invention

The subject invention pertains to materials and methods for producing plants that are resistant to infection by geminiviruses and other related viruses. Methods of the invention comprise transforming a plant with a polynucleotide wherein when the polynucleotide is

expressed in the plant, the transformed plant exhibits resistance to infection when challenged with a plant virus. In a preferred embodiment, a plant is transformed with a polynucleotide encoding a Rep protein or a mutated Rep protein derived from tomato mottle geminivirus or from tomato yellow leaf curl virus (TYLCV-Is). The methods of the invention can be used to provide resistance to viral infection in plants such as tomato and tobacco.

The subject invention also concerns polynucleotides that encode the Rep protein and mutated Rep proteins of the invention. The mutated Rep proteins are also an object of the present invention.

The present invention also concerns transformed and transgenic plants and plant tissue that contain or express a polynucleotide encoding a Rep protein or a mutated Rep protein.

Brief Description of the Drawings

Figures 1A and 1B show a Field Resistance Trial conducted in Fall 1997. **Figure 1A** shows disease progress curves of ToMoV in 'Agriset 761' and 6 tomato lines transformed with ToMoV *Rep* gene. **Figure 1B** shows the mean number of immature whiteflies per ten terminal leaflets.

Figures 2A and 2B show a Field Resistance Trial conducted in Spring, 1998. **Figure 2A** shows disease progress curves of ToMoV in 'Agriset 761', FL 7324, FL 7613 and 4 tomato lines transformed with ToMoV *Rep* gene. **Figure 2B** shows the mean number of immature whiteflies per ten terminal leaflets.

Figures 3A and 3B show a Field Resistance Trial conducted in Fall 1998. **Figure 3A** shows disease progress curves of ToMoV in 'Agriset 761', FL 7324, FL 7613, and 5 tomato lines transformed with ToMoV *Rep* gene. **Figure 3B** shows the mean number of immature whiteflies per ten terminal leaflets.

Detailed Description of the Invention

The subject invention concerns the use of a plant virus gene to transform a plant or plant tissue to confer resistance in the plant or plant tissue to infection from a plant virus. The methods of the subject invention can be used to confer resistance in a plant to infection by a plant pathogen such as, for example, a geminivirus. The method comprises transforming a plant with a polynucleotide such that when the polynucleotide is expressed in the plant the plant then exhibits resistance to infection by plant viruses. In one embodiment of the invention, a plant is transformed by wounding and agroinfection with an *Agrobacterium* containing a polynucleotide of the invention that is transferred to the plant upon agroinfection of the plant. Preferably, the polynucleotide used in the methods of the invention encodes a plant virus Rep protein or a mutant Rep protein, or a fragment or variant thereof. In an exemplified embodiment, the polynucleotide encodes a Rep protein of tomato mottle geminivirus (ToMoV). The nucleotide sequence of a ToMoV (component A) virus is disclosed in Genbank having accession number L14460. Abouzid *et al.* (1992) disclose the nucleotide sequence of the ToMoV *Rep* gene (referred to therein as AL1 and corresponding to nucleotides 1523 to 7 of the sequence shown in Figure 1 of Abouzid *et al.* (1992)). In another embodiment, the polynucleotide encodes a tomato yellow leaf curl virus (TYLCV-Is) Rep protein. The nucleotide sequences of several TYLCV-Is viral isolates are disclosed in Genbank, including isolates from Israel (accession number X15656), Cuba (accession number AJ223505), Dominican Republic (accession number AF024715), Egypt (accession number L12219), Jamaica (accession number U84146), Lebanon (accession number AF160875), Mexico (accession number AF168709) and Spain (accession number AJ223505).

In a preferred embodiment of the invention, a virus-resistant transgenic plant line prepared according to the methods described herein is crossed with a transgenic plant line that is resistant to the same virus and derived from a different transformation event to produce hybrids that exhibit increased virus resistance over the parent lines.

The methods of the subject invention can be used to confer resistance in plants to infection by viruses such as geminiviruses, and include, for example, tomato mottle virus, cabbage leaf curl geminivirus, potato yellow mosaic virus, tomato golden mosaic virus, tomato yellow mosaic virus, tomato leaf crumple virus, tomato yellow leaf curl virus, pepper huasteco virus and others. Plants which can be transformed according to the methods of the subject invention include, but are not limited to, tomato and tobacco.

The subject invention also concerns polynucleotide molecules that encode modified or mutated forms of a plant virus Rep protein which when expressed in a plant confers resistance to infection by plant viruses. In one embodiment, the polynucleotide encodes a Rep protein of ToMoV or TYLCV-Is. Modifications and mutations contemplated within the scope of the invention include Rep proteins comprising amino acid substitutions, deletions, and insertions. Also contemplated within the scope of the invention are Rep polypeptides containing the mutations in the amino acid sequence.

The subject invention also concerns recombinant polynucleotide molecules comprising a vector in which a polynucleotide sequence encoding a plant virus Rep protein, or a mutant thereof, which is expressible in a suitable host plant has been inserted. Suitable vectors may be selected from those known in the art including plasmids, phage DNA, or derivatives or fragments thereof, or combinations of plasmids and phage DNA, and yeast plasmids. The polynucleotide encoding the Rep protein can be inserted into the multiple cloning site of a vector, such as the commercially available pUC vectors or the pGEM vectors, which allow for the excision of the polynucleotide having restriction termini adapted for insertion into any desirable plant expression or integration vector. In addition, regulatory sequences such as promoters can be operatively linked to the coding sequences of the polynucleotides of the present invention. For example, the 35S promoter of cauliflower mosaic viruses (CaMV) can be used with the subject invention. Other plant expression vectors can also be used in the present invention.

The present invention also concerns cells infected, transformed, or transfected with a polynucleotide of the present invention that encodes a Rep protein or a mutated Rep

protein. Preferably, the Rep protein or mutant thereof is derived from ToMoV or TYLCV-Is. In one embodiment, the polynucleotide is inserted into a suitable vector, and the recombinant vector is used to transform a bacterium or other host which can then be used to introduce the polynucleotide into a plant cell. Suitable hosts that can be infected, transformed, or transfected with the polynucleotide of the invention include gram positive and gram negative bacteria such as *E. coli* and *Bacillus subtilis*. Other suitable hosts include *Agrobacterium* cells, insect cells, plant cells, and yeast cells. *Agrobacterium* containing the polynucleotide of the invention can be used to transform plant cells with the polynucleotide according to standard methods known in the art. Polynucleotides can also be introduced into plant cells by a biolistic method (Carrer, 1995) and other methods known in the art.

The subject invention also concerns transformed and transgenic plants and plant tissue, including plant seeds, that exhibit resistance to infection by plant geminiviruses such as ToMoV and the like. In one embodiment, a transformed or transgenic plant of the invention comprises a polynucleotide that encodes a Rep protein or a mutated Rep protein. Preferably, the Rep protein or mutated Rep protein is derived from ToMoV or TYLCV-Is. Transformed and transgenic plants and plant tissue of the invention can be prepared from plants such as tomato, tobacco and others.

As those of ordinary skill in the art will appreciate, any number of different nucleotide sequences can be used, based on the degeneracy of the genetic code, to encode a Rep protein or a mutated Rep protein of the present invention. Accordingly, any polynucleotide sequence which encodes a Rep protein or mutated Rep protein, or a fragment or variant thereof, falls within the scope of this invention.

Two hybrid parent tomato lines (from J.W. Scott) FL 7324 and FL 7613, were transformed with the ToMoV *Rep* gene in the sense orientation. Both tolerance and immunity to ToMoV were seen in plants containing the transgene in T₁ through T₄ generations. Preliminary Southern analysis has indicated that resistant plants have either one or two genes. Resistance has been evaluated in the field in the fall and spring seasons of 1996, 1997, and 1998. Plants in the field were selected for resistance and horticultural

qualities. Yields of transformed plants were equivalent to non-transformed plants in the absence of virus, and were significantly greater in the presence of ToMoV. Transformed plants appeared to have high levels of tolerance to ToMoV.

Resistance to infection was evaluated by simulating natural inoculation as much as possible. Other laboratories use such techniques as biolistic and Agro-inoculation, which never occur naturally, and bypass the normal modes of entry into the plant cell where resistance mechanisms may exist. The inoculation described herein is a simulation of a worst case scenario in a transplant house or a grower's field. Plants are inoculated at an early stage in development, when plants are highly attractive to whiteflies and are highly susceptible to infection by ToMoV. Whiteflies are reared on virus-infected tomato plants, which eliminates the interference of whitefly feeding preferences, and is similar to inoculation by viruliferous whiteflies in the field (Polston *et al.*, 1996). This inoculation protocol results in an inoculation efficiency of 100%.

All patents, patent applications, provisional applications, and publications referred to or cited herein are incorporated by reference in their entirety to the extent they are not inconsistent with the explicit teachings of this specification.

Following are examples which illustrate procedures for practicing the invention. These examples should not be construed as limiting. All percentages are by weight and all solvent mixture proportions are by volume unless otherwise noted.

Example 1 — Field Evaluation of Transgenic Resistance to ToMoV

Advanced breeding lines, FL 7324 and FL 7613 were transformed with ToMoV *Rep* gene using standard *Agrobacterium*-mediated transformation techniques. A vector comprising a 35S CaMV (non-enhanced) promoter linked to the *Rep* gene was used in the transformation. Plant tissue was wounded using tungsten. Plants that contained a *Rep* transgene were identified using PCR methods. Those plants were then evaluated for resistance to viral infection. Untransformed parents and transformed lines from three

different transformation events were evaluated for resistance to ToMoV and for yield, both in the presence and absence of ToMoV. Lines shown in the following tables are four generations past transformation. Lines 02 and 04 are from the same T₀ plant in a FL 7613 background, lines 09 and 10 are from a second T₀ plant and their background is FL 7324, and lines 11 and 12 are from a third T₀ plant, and their background is FL 7613.

Southern analysis using two restriction enzymes, one which cut inside the transgene and one which cut outside the transgene, of several lines of Rep-transformed tomatoes revealed that the ToMoV resistance in 4 of the R4 generation lines (lines 02, 04, 11, 12) appeared to be due to one insertion site and one copy of the transgene. Multiple copies were present in resistant lines 09 and 10. Lines 02 and 04, 11 and 12, and 09 and 10 were the result of three different transformations.

Example 2 — Performance in the Presence of ToMoV and Whiteflies

For three seasons, Fall 1997, Spring 1998, and Fall 1998, tomato transplants to be evaluated were set into a field which was within 20 feet of a large block of tomatoes which was a continuous source of viruliferous whiteflies throughout the season. No imidacloprid was applied to the plants being evaluated but attempts were made to keep whitefly populations below a threshold which would result in irregular ripening of the fruit (20 immature whitefly/10 terminal leaflets). Whitefly populations were evaluated approximately every 2 week beginning about 4 week after transplanting. Whitefly populations varied each season, with the highest populations occurring in the Fall 1998 trial. The trials consisted of 15 plants per block, with three replications, in a randomized complete block design. Plants were evaluated every other week for the presence of whiteflies and virus. Plants displaying virus-like symptoms were assayed by nucleic acid hybridization to confirm the presence of ToMoV. Fruit were harvested from plants in two pickings, graded, and marketable yields were calculated.

Example 3 — Yields

Results are shown in Tables 1, 2 and 3. The transformed lines yielded as much or more than the untransformed parents and the commercial hybrid 'Agriset' in all three trials. The best transformed lines, 02, 04, 11 and 12 yielded approximately 50% - 100% more marketable fruit than the untransformed lines. Yields of these transformed lines in the presence of ToMoV and whiteflies were comparable to yields of the untransformed lines in the absence of virus and whiteflies. In addition, transformed plants yielded well in both fall and spring production seasons.

Example 4 — ToMoV Resistance

Infection rates as determined by viral nucleic acid detection, were much lower in all transformed lines than in untransformed lines. Transformed lines has high levels of tolerance, which were overcome only with high populations of viruliferous whiteflies. Figures 1A, 2A, and 3A show the disease progress curves from untransformed and transformed lines from trials over three seasons. The highest rates of infection were observed in the Fall 1998 season (Figures 3A and 3B) which had extremely high populations of viruliferous whiteflies (at 100 per 10 terminal leaflets).

Even with those unusually high populations, transformed lines though infected produced yields similar to those plants not exposed to virus (Tables 3 and 6). Symptoms in infected transformed plants were milder than those of infected untransformed plants.

Example 5 — Performance in the absence of ToMoV and Whiteflies

For three seasons, Fall 1997, Spring 1998, and Fall 1998, tomato transplants to be evaluated were set into a field which was not located near a source of ToMoV or whiteflies. Imidacloprid was applied at the time of transplant to the field, and plants were monitored weekly for whiteflies. When whiteflies were detected (about the 6 to 8 week after transplant) plants were sprayed with a rotation of insecticides to manage whitefly populations. This resulted in less than 0.1% infection of ToMoV in these plants, and allowed an evaluation of

yields without the influence of virus. The trials consisted of 15 plants per block, with three replications, in a randomized complete block design. Plants displaying virus-like symptoms were assayed by nucleic acid hybridization to confirm the presence of ToMoV. Fruit were harvested from plants in two pickings, graded, and marketable yields were calculated.

5

Example 6 — Yield

Marketable yields of untransformed and transformed plants are shown in Tables 4, 5, and 6. Yields of transformed lines were either significantly greater (Table 4) or not significantly different to those of the transformed plants. The best yielding transformed lines were 02, 04, 11 and 12 which yielded as good or better than their untransformed parent, FL 7613 in the absence of ToMoV infection.

10

Example 7 — Hybrid Transgenic Tomatoes

Hybrid tomatoes were made by crossing transgenic lines with the untransformed genotype and between transgenic lines derived from different transformation events. It was found that several of the hybrids of different transgenic lines were more resistant to ToMoV than either open-pollinated parent. This is known as pyramiding of resistance genes and resulted in improved resistance of the transgenic plants to infection.

15

Transgenic lines 02 and 11 were crossed and their hybrid progeny was evaluated for yield and ToMoV resistance. An increase in resistance was observed in the hybrid. ToMoV resistance in the hybrids was superior to both the transformed parents and nontransformed parents (Table 7). Infection of the hybrid was 1/3 that of the transgenic parent and 1/10 that of the untransformed parent. Resistance appeared to be additive. Yields of these crosses are currently being analyzed but are expected to be high based on previous results with the untransformed parents. This data shows that hybridizing transgenic parents is a method to improve geminivirus resistance.

20

25

Tables 1-3 show a comparison of yields of ToMoV *Rep* - transformed tomatoes with untransformed parents and 'Agriset' in the presence of ToMoV and whiteflies.

Tables 4-6 show a comparison of yields of ToMoV *Rep* - transformed tomatoes with untransformed parents in the absence of ToMoV and whiteflies.

Table 1. Fall 1997 Trial				
Line	Total Marketable Yield (ca/A)	Extra Large Fruit (ca/A)	Avg. Fruit Size (lbs)	Culls (ca/A)
02	1556.5 a	766.7 a	0.33 ab	580.8 a
04	1695.9 a	952.5 a	0.38 a	580.8 a
09	952.5 b	92.9 c	0.27 c	511.1 a
10	859.6 b	69.7 c	0.28 c	464.6 a
FL 7324	-	-	-	-
FL 7613	-	-	-	-
Agriset	952.5 b	325.2 b	0.31 bc	325.2 b

Table 2. Spring 1998 Trial				
Line	Total Marketable Yield (ca/A)	Extra Large Fruit (ca/A)	Avg. Fruit Size (lbs)	Culls (ca/A)
02	1498.5 a	1028.9 a	0.394 a	627.3 a
04	1237.1 ab	923.5 a	0.373 a	697.0 a
09	1359.1 ab	156.8 b	0.266 bc	592.4 a
10	1341.6 ab	174.2 b	0.261 c	609.8 a
FL 7324	906.1 ab	139.4 b	0.272 c	592.4 a
FL 7613	784.1 b	487.9 b	0.365 a	348.5 a
Agriset	714.4 b	278.8 b	0.320 b	331.1 a

Table 3. Fall 1998 Trial

Line	Total Marketable Yield (ca/A)	Extra Large Fruit (ca/A)	Avg. Fruit Size (lbs)	Culls ¹ (ca/A)
04	1702.9 a ²	1247.6 a	0.377 a	480.0 ab
10	759.7 b	30.2 b	0.240 d	736.5 a
11	1689.0 a	999.0 a	0.358 ab	573.8 ab
12	1905.0 a	1191.8 a	0.356 ab	573.8 ab
FL 7324	325.2 b	23.2 b	0.284 cd	401.9 b
FL 7613	727.2 b	464.6 b	0.377 ab	471.6 ab
Agriset	580.8 b	255.6 b	0.323 bc	325.3 b

¹Culls include all fruit rated not marketable (includes insect damage, disease, irregular shape, etc.)

² Letters after values denote significant differences as determined by Duncan's Multiple Range.

Table 4. Fall 1997 Trial

Line	Total Marketable Yield (ca/A)	Extra Large Fruit (ca/A)	Avg. Fruit Size (lbs)	Culls (ca/A)
02	1359.1 a	731.8 a	0.35 ab	609.8 a
04	1184.8 ab	662.1 a	0.35 ab	592.4 a
09	906.1 bc	104.5 c	0.27 c	400.8 a
10	609.8 cd	22.7 c	0.26 c	435.6 ab
FL 7324	993.2 b	278.8 bc	0.32 b	313.6 b
FL 7613	592.4 d	435.6 ab	0.37 a	278.8 b

Table 5. Spring 1998 Trial				
Line	Total Marketable Yield (ca/A)	Extra Large Fruit (ca/A)	Avg. Fruit Size (lbs)	Culls (ca/A)
02	1968.9 a	1219.7 ab	0.36 b	784.1 a
04	2108.3 a	1515.9 ab	0.37 b	784.1 a
09	1986.3 a	296.2 c	0.29 c	540.1 ab
10	2265.1 a	278.8 c	0.26 d	418.2 ab
FL 7324	2456.8 a	906.0 bc	0.30 a	278.8 b
FL 7613	2317.4 a	1812.1 a	0.40 a	435.6 ab
Agriset	2352.2 a	1550.7 ab	0.36 b	313.6 b

Table 6. Fall 1998 Trial				
Line	Total Marketable Yield (ca/A)	Extra Large Fruit (ca/A)	Avg. Fruit Size (lbs)	Culls ¹ (ca/A)
02	1568.2 abc ²	911.3 b	0.348 b	412.9 a
04	1503.7 abc	1050.7 b	0.368 b	423.4 a
09	744.0 d	12.2 c	0.240 d	639.5 a
10	998.4 cd	64.5 c	0.272 c	557.6 a
11	1747.6 ab	1115.1 ab	0.351 b	597.6 a
12	1742.4 ab	1197.0 ab	0.361 ab	522.7 a
FL 7324	1184.8 bcd	174.2 c	0.270 c	418.2 a
FL 7613	1951.5 a	1510.7 a	0.388 a	418.2 a
Agriset	1381.7 abcd	876.4 d	0.360 ab	505.3 a

¹Culls include all fruit rated not marketable (includes insect damage, disease, irregular shape, etc.)

² Letters after values denote significant differences as determined by Duncan's Multiple Range.

Table 7. Evaluation of Resistance to ToMoV in a Hybrid of Two Transgenic Lines - Fall 1998 Trial		
Line	Transformation Status	Incidence of ToMoV (60 days post transp.) ¹
FL 7324	not transformed	100%
FL 7613	not transformed	100%
F97/02	FL 7613- Rep	36.7%
F97/11	FL 7324 - Rep	33.3%
F ₁	F97/0202 x F97/11	12%

It should be understood that the examples and embodiments described herein are for illustrative purposes only and that various modifications or changes in light thereof will be suggested to persons skilled in the art and are to be included within the spirit and purview of this application and the scope of the appended claims.

References

- 5 Abouzid, A.M., J.E. Polston, and E. Hiebert (1992) "The nucleotide sequence of tomato mottle virus, a new geminivirus isolated from tomato in Florida" *J. Gen. Virology* 73:3225-3229.
- 10 Abouzid, A., J.E. Polston, W.B. Hunter, E. Hiebert (1996) "Modified coat protein of tomato mottle geminivirus confers resistance in transgenic tobacco" *Phytopathology* 86(11):593 Suppl. Abstract No. 832A.
- 15 Brown, J.K., J. Bird, G. Banks, M. Sosa, K. Kiesler, I Cabrera, G. Fornaris (1995) "First report of a whitefly-transmitted geminivirus epidemic in tomato in Puerto Rico" *Plant Disease* 79:1250.
- 20 Brunetti, A., M. Tavazza, E. Noris, R. Tavazza, P. Caciagli, S. Crespi, G.P. Accotto (1997) "High expression of truncated viral Rep protein confers resistance to tomato yellow leaf curl virus in transgenic tomato plants" *Mol. Plant-Microbe Interact.* 10:571-579.
- 25 Cahill, M., K. Gorman, S. Kay, I. Denholm (1996) "Baseline determination and detection of resistance to imidacloprid in *Bemisia tabaci* (Homoptera:Aleyrodidae)" *Bull. of Ento. Res.* 86:343-349.
- 30 Carrer, H., P. Maliga (1995) "Targeted insertion of foreign genes into the tobacco plastid genium without physical linkage to the selectable marker" *Biotechnology* 13:791-794.
- 35 Duan, Y.-P., C.A. Powell, S.E. Webb, D.E. Purcifull, E. Hiebert (1997a) "Geminivirus resistance in transgenic tobacco expressing mutated BC1 protein" *MPMI* 10:617-623.
- Duan, Y.-P., C.A. Powell, D.E. Purcifull, P. Broglio, E. Hiebert (1997b) "Phenotypic variation in transgenic tobacco expressing mutated geminivirus movement/pathogenicity (BC1) proteins" *MPMI* 10:1065-1074.
- Hanson, S.F., R.A. Hoogstraten, P. Ahlquist, R.L. Gilbertson, D.R. Russell, D.P. Maxwell (1995) "Mutational analysis of a putative NTP-binding domain in the replication-associated protein (AC1) of bean golden mosaic geminivirus" *Virology* 211:1-9.

- Hong, Y. and J. Stanley (1996) "Virus resistance in *Nicotiana benthamiana* conferred by African cassava mosaic virus replication-associated protein (AC1) transgene" *Mol. Plant-Microbe Interact.* 9:219-225.
- 5 Noris, E., G.P. Accotto, R. Tavazza, A. Brunetti, S. Crespi, M. Tavazza (1996) "Resistance to tomato yellow leaf curl geminivirus in *Nicotiano benthamiana* plants transformed with a truncated viral C1 gene" *Virology* 224:130-138.
- 10 Polston, J.E., D.O. Chellemi, D.J. Schuster, R.J. McGovern, P.A. Stansly (1996) "Spatial and temporal dynamics of tomato mottle geminivirus and *Bemisia tabaci* in Florida tomato fields" *Plant Disease* 80:1022-1028.
- 15 Polston, J.E. and P.K. Anderson (1997) "The emergence of whitefly-transmitted geminiviruses in tomato in the Western hemisphere" *Plant Disease* 81:1358-1369.
- Polston, J.E., R.J. McGovern, L.G. Brown (1999) "The appearance of tomato yellow leaf curl virus in Florida and implications for the spread of the and other geminiviruses in the U.S." *Plant Dis.* 83:984-988.
- 20 Sinisterra, X., J.E. Polston, E. Hiebert, A. Abouzid (1997) "RNA-mediated virus resistance in tobacco plants transformed with a modified coat protein of tomato mottle geminivirus" *Phytopathology* 87:S91.
- 25 Sinisterra, X.H., J.E. Polston, A.M. Abouzid, E. Hiebert (1999) "Tobacco Plants Transformed with a Modified Coat Protein of Tomato Mottle Begomovirus Show Resistance to Virus Infection" *Phytopathology* 89(8):701-706.
- 30 Stout, J.T., H.T. Lui, J.E. Polston, R.L. Gilbertson, M.K. Nakhia, S.F. Hanson, D.P. Maxwell (1997) "Engineered rep gene-mediated resistance to tomato mottle geminivirus in tomato" *Phytopathology* 87:S96.
- Williams, L., T.J. Dennehy, J.C. Palumbo (1996) "Development of a resistance management program for imidacloprid" *Proc. Beltwide Cotton Conferences* pp. 752-755.

Claims

We claim:

- 1 1. A method for providing resistance to infection by a plant virus in a plant or plant
2 tissue, said method comprising transforming said plant or plant tissue with a polynucleotide
3 that encodes a Rep protein, or a fragment or variant thereof, of said plant virus.
- 1 2. The method according to claim 1, wherein said plant virus is a geminivirus.
- 1 3. The method according to claim 1, wherein said geminivirus is selected from the
2 group consisting of tomato mottle virus, cabbage leaf curl geminivirus, potato yellow mosaic
3 virus, tomato golden mosaic virus, tomato yellow mosaic virus, tomato leaf crumple virus,
4 tomato yellow leaf curl virus and pepper huasteco virus.
- 1 4. The method according to claim 1, wherein said polynucleotide encodes a Rep
2 protein of a tomato mottle geminivirus.
- 1 5. The method according to claim 1, wherein said polynucleotide encodes a Rep
2 protein of a tomato yellow leaf curl virus (TYLCV-Is).
- 1 6. The method according to claim 1, wherein said plant or plant tissue is tomato or
2 tobacco.
- 1 7. The method according to claim 1, wherein said plant or plant tissue is transformed
2 with said polynucleotide by agroinfection.
- 1 8. The method according to claim 1, wherein said plant or plant tissue is transformed
2 with said polynucleotide by biolistic targeting.

1 9. A transgenic plant or plant tissue having increased resistance to infection by a
2 plant virus, wherein said plant or plant tissue comprises a polynucleotide sequence that
3 encodes a plant virus Rep protein, or a fragment or variant thereof.

1 10. The transgenic plant or plant tissue according to claim 9, wherein said plant or
2 plant tissue is tomato or tobacco.

1 11. The transgenic plant or plant tissue according to claim 9, wherein said
2 polynucleotide encodes a Rep protein of a tomato mottle virus.

1 12. The transgenic plant or plant tissue according to claim 9, wherein said
2 polynucleotide encodes a Rep protein of a tomato yellow leaf curl virus (TYLCV-Is).

1 13. The transgenic plant or plant tissue according to claim 9, wherein said plant
2 tissue is a plant seed.

1 14. The transgenic plant or plant tissue according to claim 9, wherein said transgenic
2 plant or plant tissue is crossed with a second transgenic plant or plant tissue that is resistant
3 to said plant virus and derived from a distinct transformation event, producing a hybrid plant
4 or plant tissue that exhibits increased resistance to infection by said plant virus.

1 15. A cell transformed with a polynucleotide sequence that encodes a plant virus Rep
2 protein, or a fragment or variant thereof.

1 16. The transformed cell according to claim 15, wherein said polynucleotide encodes
2 a Rep protein of a tomato mottle virus.

1 17. The transformed cell according to claim 15, wherein said polynucleotide encodes
2 a Rep protein of a tomato yellow leaf curl virus (TYLCV-Is).

1 18. The transformed cell according to claim 15, wherein said cell is selected from
2 the group consisting of bacterial cell, insect cell, plant cell and yeast cell.

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65
66
67
68
69
70
71
72
73
74
75
76
77
78
79
80
81
82
83
84
85
86
87
88
89
90
91
92
93
94
95
96
97
98
99
100
101
102
103
104
105
106
107
108
109
110
111
112
113
114
115
116
117
118
119
120
121
122
123
124
125
126
127
128
129
130
131
132
133
134
135
136
137
138
139
140
141
142
143
144
145
146
147
148
149
150
151
152
153
154
155
156
157
158
159
160
161
162
163
164
165
166
167
168
169
170
171
172
173
174
175
176
177
178
179
180
181
182
183
184
185
186
187
188
189
190
191
192
193
194
195
196
197
198
199
200
201
202
203
204
205
206
207
208
209
210
211
212
213
214
215
216
217
218
219
220
221
222
223
224
225
226
227
228
229
230
231
232
233
234
235
236
237
238
239
240
241
242
243
244
245
246
247
248
249
250
251
252
253
254
255
256
257
258
259
260
261
262
263
264
265
266
267
268
269
270
271
272
273
274
275
276
277
278
279
280
281
282
283
284
285
286
287
288
289
290
291
292
293
294
295
296
297
298
299
300
301
302
303
304
305
306
307
308
309
310
311
312
313
314
315
316
317
318
319
320
321
322
323
324
325
326
327
328
329
330
331
332
333
334
335
336
337
338
339
340
341
342
343
344
345
346
347
348
349
350
351
352
353
354
355
356
357
358
359
360
361
362
363
364
365
366
367
368
369
370
371
372
373
374
375
376
377
378
379
380
381
382
383
384
385
386
387
388
389
390
391
392
393
394
395
396
397
398
399
400
401
402
403
404
405
406
407
408
409
410
411
412
413
414
415
416
417
418
419
420
421
422
423
424
425
426
427
428
429
430
431
432
433
434
435
436
437
438
439
440
441
442
443
444
445
446
447
448
449
450
451
452
453
454
455
456
457
458
459
460
461
462
463
464
465
466
467
468
469
470
471
472
473
474
475
476
477
478
479
480
481
482
483
484
485
486
487
488
489
490
491
492
493
494
495
496
497
498
499
500
501
502
503
504
505
506
507
508
509
510
511
512
513
514
515
516
517
518
519
520
521
522
523
524
525
526
527
528
529
530
531
532
533
534
535
536
537
538
539
540
541
542
543
544
545
546
547
548
549
550
551
552
553
554
555
556
557
558
559
560
561
562
563
564
565
566
567
568
569
570
571
572
573
574
575
576
577
578
579
580
581
582
583
584
585
586
587
588
589
590
591
592
593
594
595
596
597
598
599
600
601
602
603
604
605
606
607
608
609
610
611
612
613
614
615
616
617
618
619
620
621
622
623
624
625
626
627
628
629
630
631
632
633
634
635
636
637
638
639
640
641
642
643
644
645
646
647
648
649
650
651
652
653
654
655
656
657
658
659
660
661
662
663
664
665
666
667
668
669
670
671
672
673
674
675
676
677
678
679
680
681
682
683
684
685
686
687
688
689
690
691
692
693
694
695
696
697
698
699
700
701
702
703
704
705
706
707
708
709
710
711
712
713
714
715
716
717
718
719
720
721
722
723
724
725
726
727
728
729
730
731
732
733
734
735
736
737
738
739
740
741
742
743
744
745
746
747
748
749
750
751
752
753
754
755
756
757
758
759
760
761
762
763
764
765
766
767
768
769
770
771
772
773
774
775
776
777
778
779
780
781
782
783
784
785
786
787
788
789
790
791
792
793
794
795
796
797
798
799
800
801
802
803
804
805
806
807
808
809
810
811
812
813
814
815
816
817
818
819
820
821
822
823
824
825
826
827
828
829
830
831
832
833
834
835
836
837
838
839
840
841
842
843
844
845
846
847
848
849
850
851
852
853
854
855
856
857
858
859
860
861
862
863
864
865
866
867
868
869
870
871
872
873
874
875
876
877
878
879
880
881
882
883
884
885
886
887
888
889
890
891
892
893
894
895
896
897
898
899
900
901
902
903
904
905
906
907
908
909
910
911
912
913
914
915
916
917
918
919
920
921
922
923
924
925
926
927
928
929
930
931
932
933
934
935
936
937
938
939
940
941
942
943
944
945
946
947
948
949
950
951
952
953
954
955
956
957
958
959
960
961
962
963
964
965
966
967
968
969
970
971
972
973
974
975
976
977
978
979
980
981
982
983
984
985
986
987
988
989
990
991
992
993
994
995
996
997
998
999
1000
1001
1002
1003
1004
1005
1006
1007
1008
1009
1010
1011
1012
1013
1014
1015
1016
1017
1018
1019
1020
1021
1022
1023
1024
1025
1026
1027
1028
1029
1030
1031
1032
1033
1034
1035
1036
1037
1038
1039
1040
1041
1042
1043
1044
1045
1046
1047
1048
1049
1050
1051
1052
1053
1054
1055
1056
1057
1058
1059
1060
1061
1062
1063
1064
1065
1066
1067
1068
1069
1070
1071
1072
1073
1074
1075
1076
1077
1078
1079
1080
1081
1082
1083
1084
1085
1086
1087
1088
1089
1090
1091
1092
1093
1094
1095
1096
1097
1098
1099
1100
1101
1102
1103
1104
1105
1106
1107
1108
1109
1110
1111
1112
1113
1114
1115
1116
1117
1118
1119
1120
1121
1122
1123
1124
1125
1126
1127
1128
1129
1130
1131
1132
1133
1134
1135
1136
1137
1138
1139
1140
1141
1142
1143
1144
1145
1146
1147
1148
1149
1150
1151
1152
1153
1154
1155
1156
1157
1158
1159
1160
1161
1162
1163
1164
1165
1166
1167
1168
1169
1170
1171
1172
1173
1174
1175
1176
1177
1178
1179
1180
1181
1182
1183
1184
1185
1186
1187
1188
1189
1190
1191
1192
1193
1194
1195
1196
1197
1198
1199
1200
1201
1202
1203
1204
1205
1206
1207
1208
1209
1210
1211
1212
1213
1214
1215
1216
1217
1218
1219
1220
1221
1222
1223
1224
1225
1226
1227
1228
1229
1230
1231
1232
1233
1234
1235
1236
1237
1238
1239
1240
1241
1242
1243
1244
1245
1246
1247
1248
1249
1250
1251
1252
1253
1254
1255
1256
1257
1258
1259
1260
1261
1262
1263
1264
1265
1266
1267
1268
1269
1270
1271
1272
1273
1274
1275
1276
1277
1278
1279
1280
1281
1282
1283
1284
1285
1286
1287
1288
1289
1290
1291
1292
1293
1294
1295
1296
1297
1298
1299
1300
1301
1302
1303
1304
1305
1306
1307
1308
1309
1310
1311
1312
1313
1314
1315
1316
1317
1318
1319
1320
1321
1322
1323
1324
1325
1326
1327
1328
1329
1330
1331
1332
1333
1334
1335
1336
1337
1338
1339
1340
1341
1342
1343
1344
1345
1346
1347
1348
1349
1350
1351
1352
1353
1354
1355
1356
1357
1358
1359
1360
1361
1362
1363
1364
1365
1366
1367
1368
1369
1370
1371
1372
1373
1374
1375
1376
1377
1378
1379
1380
1381
1382
1383
1384
1385
1386
1387
1388
1389
1390
1391
1392
1393
1394
1395
1396
1397
1398
1399
1400
1401
1402
1403
1404
1405
1406
1407
1408
1409
1410
1411
1412
1413
1414
1415
1416
1417
1418
1419
1420
1421
1422
1423
1424
1425
1426
1427
1428
1429
1430
1431
1432
1433
1434
1435
1436
1437
1438
1439
1440
1441
1442
1443
1444
1445
1446
1447
1448
1449
1450
1451
1452
1453
1454
1455
1456
1457
1458
1459
1460
1461
1462
1463
1464
1465
1466
1467
1468
1469
1470
1471
1472
1473
1474
1475
1476
1477
1478
1479
1480
1481
1482
1483
1484
1485
1486
1487
1488
1489
1490
1491
1492
1493
1494
1495
1496
1497
1498
1499
1500
1501
1502
1503
1504
1505
1506
1507
1508
1509
1510
1511
1512
1513
1514
1515
1516
1517
1518
1519
1520
1521
1522
1523
1524
1525
1526
1527
1528
1529
1530
1531
1532
1533
1534
1535
1536
1537
1538
1539
1540
1541
1542
1543
1544
1545
1546
1547
1548
1549
1550
1551
1552
1553
1554
1555
1556
1557
1558
1559
1560
1561
1562
1563
1564
1565
1566
1567
1568
1569
1570
1571
1572
1573
1574
1575
1576
1577
1578
1579
1580
1581
1582
1583
1584
1585
1586
1587
1588
1589
1590
1591
1592
1593
1594
1595
1596
1597
1598
1599
1600
1601
1602
1603
1604
1605
1606
1607
1608
1609
1610
1611
1612
1613
1614
1615
1616
1617
1618
1619
1620
1621
1622
1623
1624
1625
1626
1627
1628
1629
1630
1631
1632
1633
1634
1635
1636
1637
1638
1639
1640
1641
1642
1643
1644
1645
1646
1647
1648
1649
1650
1651
1652
1653
1654
1655
1656
1657
1658
1659
1660
1661
1662
1663
1664
1665
1666
1667
1668
1669
1670
1671
1672
1673
1674
1675
1676
1677
1678
1679
1680
1681
1682
1683
1684
1685
1686
1687
1688
1689
1690
1691
1692
1693
1694
1695
1696
1697
1698
1699
1700
1701
1702
1703
1704
1705
1706
1707
1708
1709
1710
1711
1712
1713
1714
1715
1716
1717
1718
1719
1720
1721
1722
1723
1724
1725
1726
1727
1728
1729
1730
1731
1732
1733
1734
1735
1736
1737
1738
1739
1740
1741
1742
1743
1744
1745
1746
1747
1748
1749
1750
1751
1752
1753
1754
1755
1756
1757
1758
1759
1760
1761
1762
1763
1764
1765
1766
1767
1768
1769
1770
1771
1772
1773
1774
1775
1776
1777
1778
1779
1780
1781
1782
1783
1784
1785
1786
1787
1788
1789
1790
1791
1792
1793
1794
1795
1796
1797
1798
1799
1800
1801
1802
1803
1804
1805
1806
1807
1808
1809
1810
1811
1812
1813
1814
1815
1816
1817
1818
1819
1820
1821
1822
1823
1824
1825
1826
1827
1828
1829
1830
1831
1832
1833
1834
1835
1836
1837
1838
1839
1840
1841
1842
1843
1844
1845
1846
1847
1848
1849
1850
1851
1852
1853
1854
1855
1856
1857
1858
1859
1860
1861
1862
1863
1864
1865
1866
1867
1868
1869
1870
1871
1872
1873
1874
1875
1876
1877
1878
1879
1880
1881
1882
1883
1884
1885
1886
1887
1888
1889
1890
1891
1892
1893
1894
1895
1896
1897
1898
1899
1900
1901
1902
1903
1904
1905
1906
1907
1908
1909
1910
1911
1912
1913
1914
1915
1916
1917
1918
1919
1920
1921
1922
1923
1924
1925
1926
1927
1928
1929
1930
1931
1932
1933
1934
1935
1936
1937
1938
1939
1940
1941
1942
1943
1944
1945
1946
1947
1948
1949
1950
1951
1952
1953
1954
1955
1956
1957
1958
1959
1960
1961
1962
1963
1964
1965
1966
1967
1968
1969
1970
1971
1972
1973
1974
1975
1976
1977
1978
1979
1980
1981
1982
1983
1984
1985
1986
1987
1988
1989
1990
1991
1992
1993
1994
1995
1996
1997
1998
1999
2000
2001
2002
2003
2004
2005
2006
2007
2008
2009
2010
2011
2012
2013
2014
2015
2016
2017
2018
2019
2020
2021
2022
2023
2024
2025
2026
2027
2028
2029
2030
2031
2032
2033
2034
2035
2036
2037
2038
2039
2040
2041
2042
2043
2044
2045
2046
2047
2048
2049
2050
2051
2052
2053
2054
2055
2056
2057
2058
2059
2060
2061
2062
2063
2064
2065
2066
2067
2068
2069
2070
2071
2072
2073
2074
2075
2076
2077
2078
2079
2080
2081
2082
2083
2084
2085
2086
2087
2088
2089
2090
2091
2092
2093
2094
2095
2096
2097
2098
2099
2100
2101
2102
2103
2104
2105
2106
2107
2108
2109
2110
2111
2112
2113
2114
2115
2116
2117
2118
2119
2120
2121
2122
2123
2124
2125
2126
2127
2128
2129
2130
2131
2132
2133
2134
2135
2136
2137
2138
2139
2140
2141
2142
2143
2144
2145
2146
2147
2148
2149
2150
2151
2152
2153
2154
2155
2156
2157
2158
2159
2160
2161
2162
2163
2164
2165
2166
2167
2168
2169
2170
2171
2172
2173
2174
2175
2176
2177
2178
2179
2180
2181
2182
2183
2184
2185
2186
2187
2188
2189
2190
2191
2192
2193
2194
2195
2196
2197
2198
2199
2200
2201
2202
2203
2204
2205
2206
2207

Abstract of the Disclosure

The subject invention pertains to materials and methods for producing plants that are resistant to infection by geminiviruses and other related viruses. Methods of the invention comprise transforming a plant with a polynucleotide wherein when the polynucleotide is expressed in the plant, the transformed plant exhibits resistance to plant viral infections. Exemplified herein is the use of a polynucleotide encoding a Rep protein derived from tomato mottle geminivirus. The methods of the invention can be used to provide resistance to viral infection in plants such as tomato and tobacco. The present invention also concerns transformed and transgenic plants in plant tissue that express a polynucleotide encoding a plant virus Rep protein, or a fragment or variant thereof.

5

10

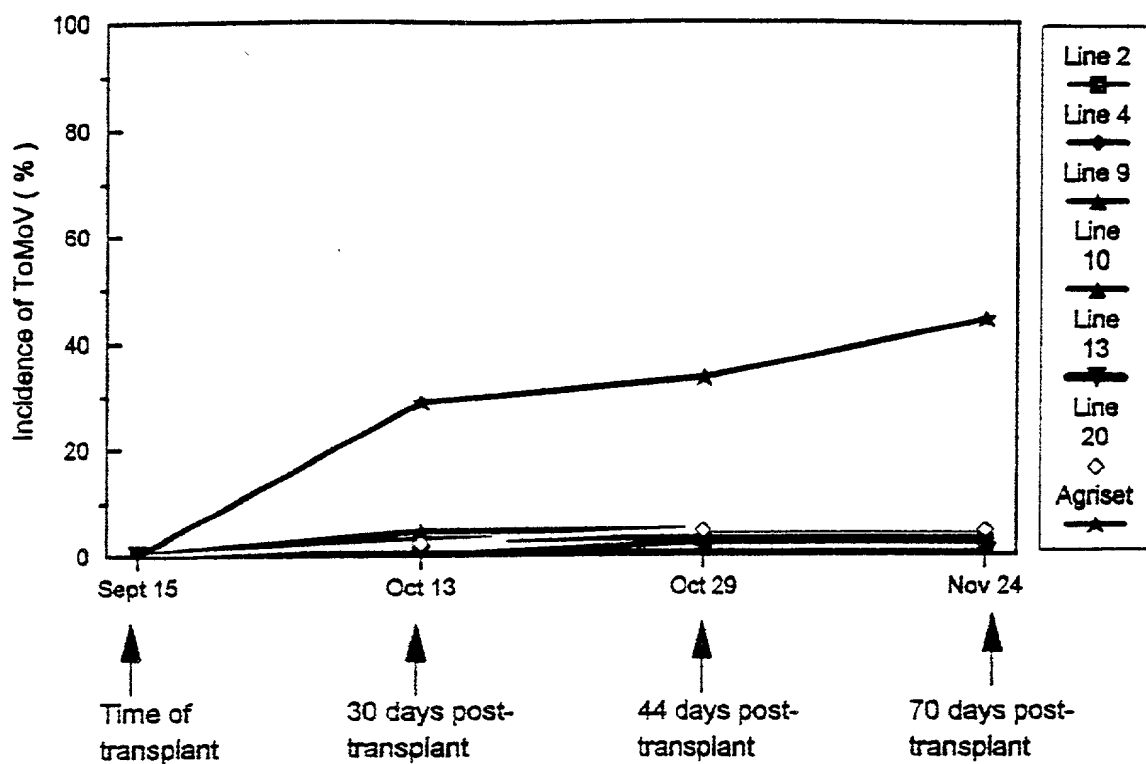
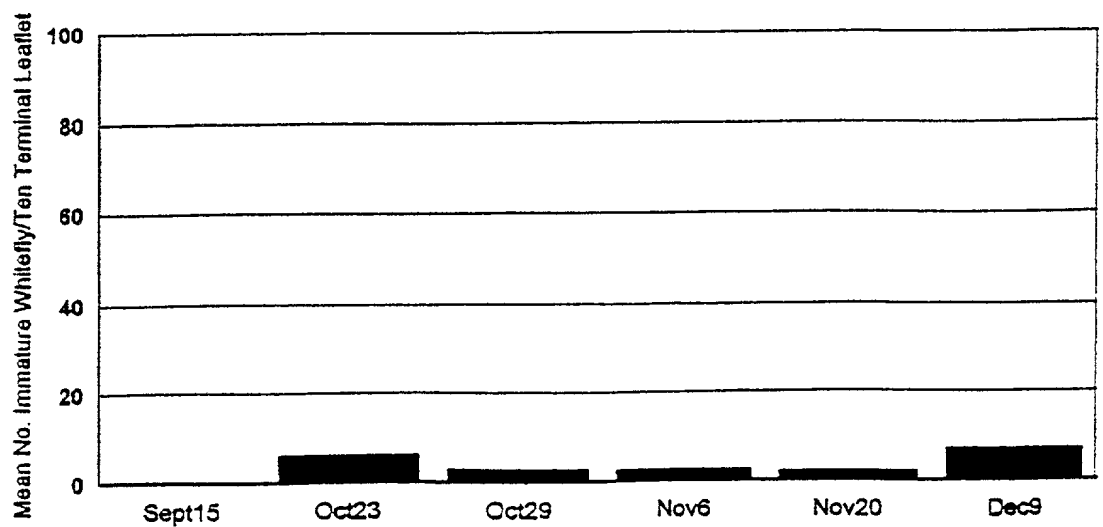


FIG. 1A



Mean Number of Immature Whiteflies per Ten Terminal Leaflets

FIG. 1B

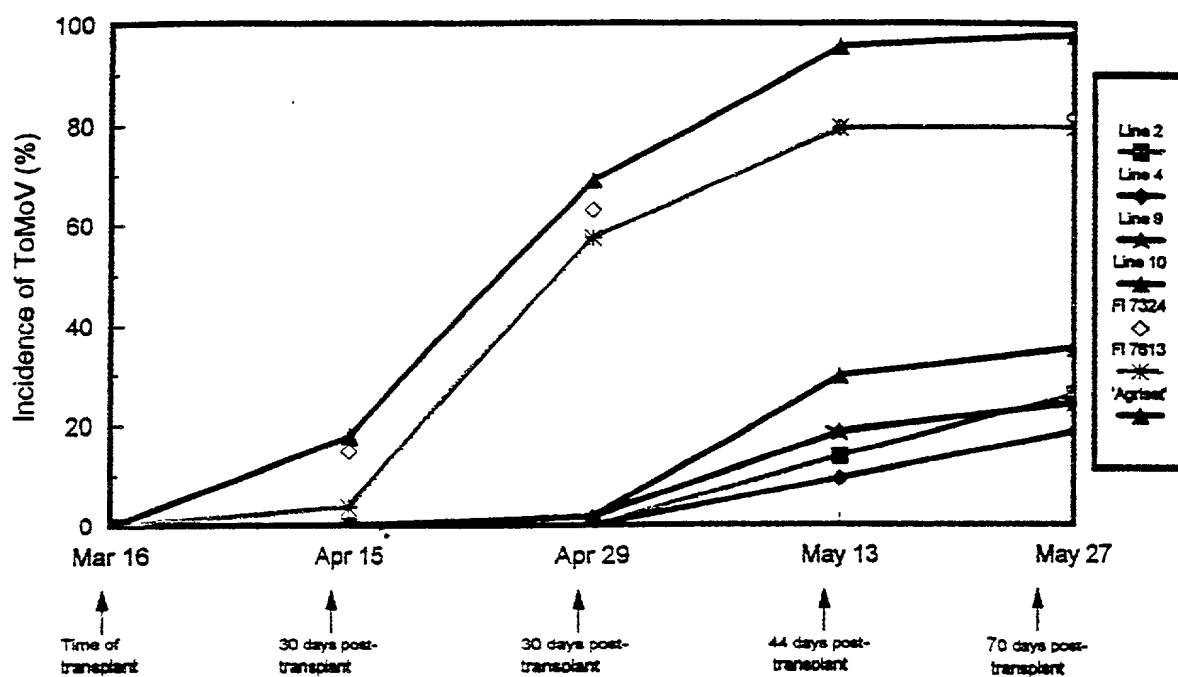
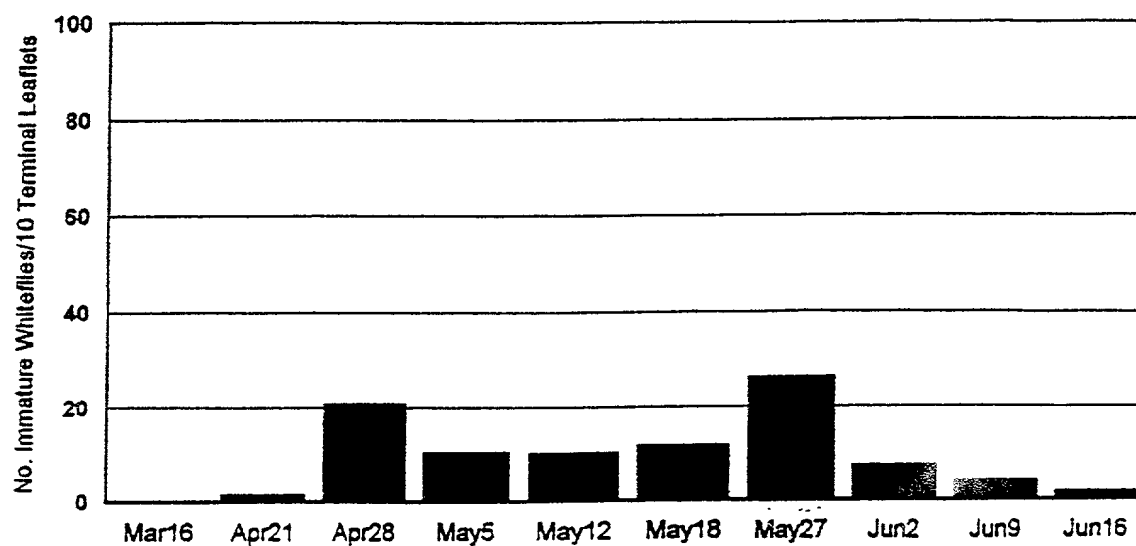


FIG. 2A



Mean Number of Immature Whiteflies per Ten Terminal Leaflets

FIG. 2B

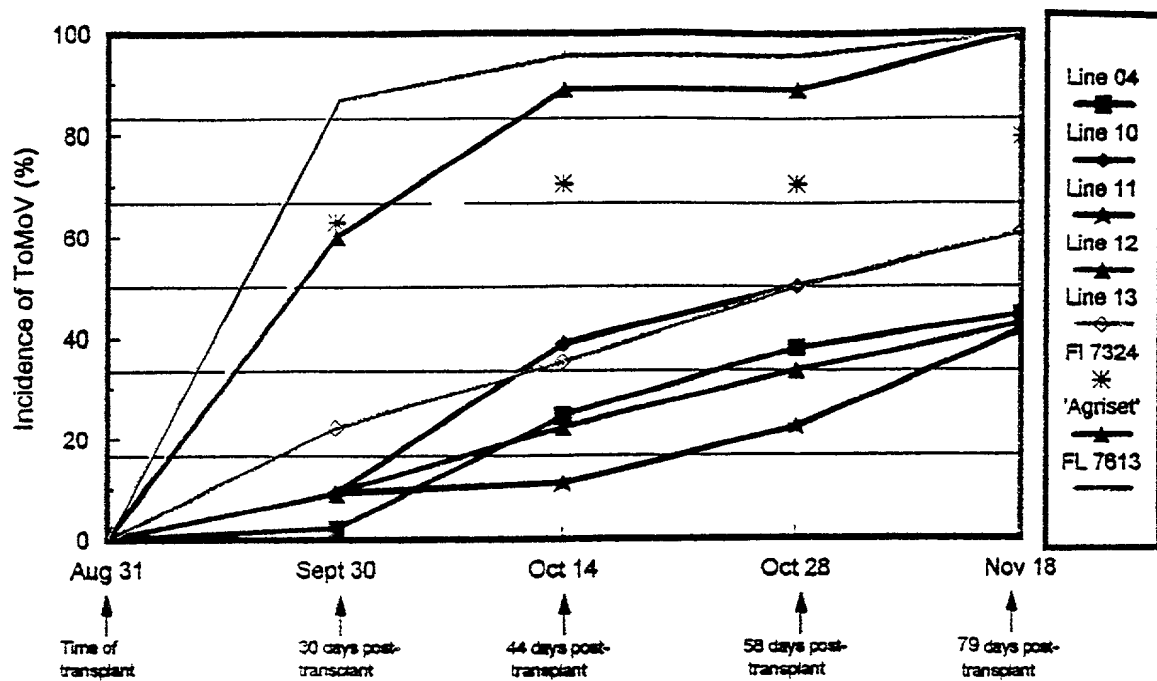
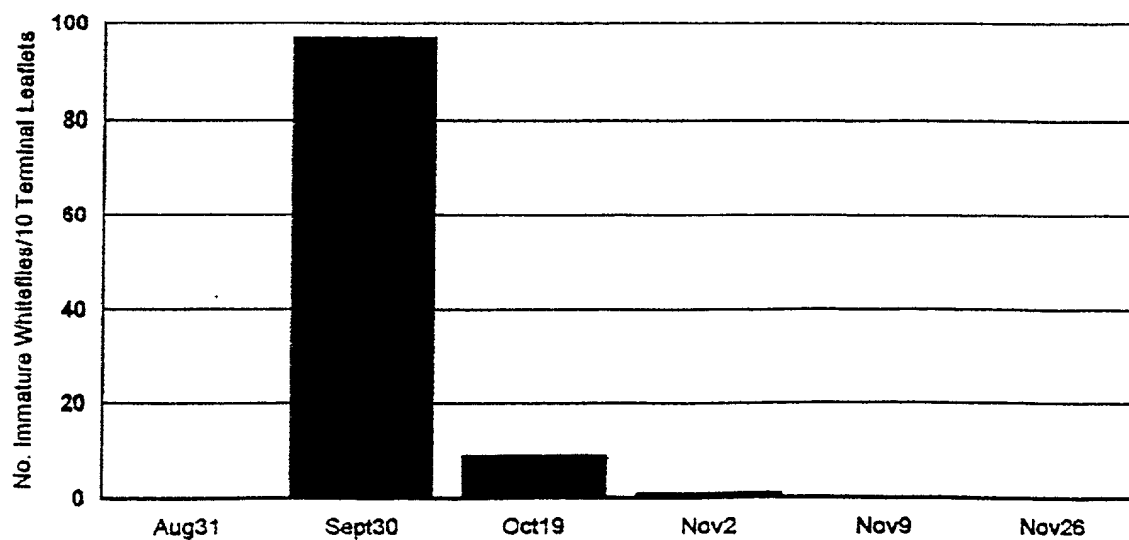


FIG. 3A



Mean Number of Immature Whiteflies per Ten Terminal Leaflets

FIG. 3B

DECLARATION (37 CFR 1.63) AND POWER OF ATTORNEY

As a below-named inventor, I hereby declare that:

My residence, post office address, and citizenship are as stated below next to my name; and

I believe that I am the original, first, and sole inventor (if only one name is listed below), or an original, first, and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled **MATERIALS AND METHODS FOR PRODUCING GEMINIVIRUS RESISTANT PLANTS** the specification for which

☒ is attached hereto.

☐ was filed _____, Serial No. _____.

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to the examination of this application in accordance with Title 37, Code of Federal Regulations, §1.56(a).

I hereby claim foreign priority benefits under Title 35, United States Code §119 and/or §365 of any foreign application(s) for patent or inventor's certificate listed below and have also identified any foreign application for patent or inventor's certificate having a filing date before that of the application on which priority is claimed:

Application Serial No.	Country	Filing Date	Priority Claimed
---------------------------	---------	-------------	------------------

I hereby claim priority benefits under Title 35, United States Code §119 of any provisional application(s) for patent listed below:

Application Serial No.	Filing Date	Priority Claimed
60/117,151	January 25, 1999	Yes

I hereby claim the benefit under Title 35, United States Code, §120 and/or §365 of any United States application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application(s) in the manner provided by the first paragraph of Title 35, United States Code, §112, I acknowledge the duty to disclose material information as defined in Title 37, Code of Federal Regulations, §1.56(a) which became available between the filing date of the prior application and the national or PCT international filing date of this application:

Application Serial No.	Filing Date	Status (patented, pending, abandoned)
---------------------------	-------------	--

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

I hereby appoint the following persons registered to practice before the Patent and Trademark Office as my attorneys with full power of substitution and revocation to prosecute this application and all divisions and continuations thereof and to transact all business in the Patent and Trademark Office connected therewith: David R. Saliwanchik, Reg. No. 31,794; Jeff Lloyd, Reg. No. 35,589; Doran R. Pace, Reg. No. 38,261; Christine Q. McLeod, Reg. No. 36,213; Jay M. Sanders, Reg. No. 39,355; James S. Parker, Reg. No. 40,119; Jean Kyle, Reg. No. 36,987; Frank C. Eisenschenk, Reg. No. 45,332; Seth M. Blum, Reg. No. P-45,489.

I request that all correspondence be sent to:

Doran R. Pace
Saliwanchik, Lloyd & Saliwanchik
2421 N.W. 41st Street, Suite A-1
Gainesville, FL 32606-6669

I further request that all telephone communications be directed to:

Doran R. Pace
352-375-8100

Name of First or Sole Inventor Jane E. Polston

Residence North Port, Florida Citizenship United States

Post Office Address 3451 Elkrem Avenue
North Port, FL 34287

Date _____

Signature of First or Sole Inventor

Name of Second Joint Inventor Ernest Hiebert

Residence Gainesville, Florida Citizenship United States

Post Office Address 2201 N.W. 36th Terrace
Gainesville, FL 32605

Date _____

Signature of Second Joint Inventor

Name of Third Joint Inventor Ahmed M. Abouzid

Residence Gainesville, Florida Citizenship Egypt

Post Office Address 3520 SW 20th Avenue, Apt. 1
Gainesville, FL 32607-4518

Date _____

Signature of Third Joint Inventor

Name of Fourth Joint Inventor Wayne B. Hunter

Residence Orlando, Florida Citizenship United States

Post Office Address 3219 S. Semoran Blvd. Apt. 327
Orlando, FL 32822

Date _____

Signature of Fourth Joint Inventor